Standard Procedures for Aquatic Animal Health Inspections

Developed and Edited by:

United States Fish and Wildlife Service
And
American Fisheries Society – Fish Health Section

Appendix 3

Position Statements

A3.1 Introduction

A. 2000 – 2002 (Initial Position Statement)

Position Statement

This chapter sums up why and how we have assembled this document. We have tried to explain the purpose and makeup of the document so there is as little confusion as possible. We had to recognize there was no way to please everyone, when so many people with differing needs and wants will use the document. We have done our best to provide what we believe is the best possible document at this time. We have adhered very closely to OIE guidelines to make this document as useful as possible for international trade inspections. We are hopeful this document will continue to grow and evolve. . Individual jurisdictions are likely to require different criteria for an aquatic animal health inspection and those criteria shall supercede the recommendations set forth in this chapter.

A3.2 Sampling

A. 2000 – 2002 (Initial Position Statement)

Position Statement

This chapter was by far the most difficult to develop, due to the vast nature of situations and scenarios an inspector might come across. It is impossible to cover all scenarios and situations; therefore while we have done our best to cover as much as we can it will remain incumbent on the inspector to determine how best to sample in any given situation. Individual jurisdictions are likely to require different criteria and those criteria shall supercede the recommendations set forth in this chapter.

A3.3 Bacteriology

A. 2000 – 2002 (Initial Position Statement)

Position Statement

The pathogens selected were those the committee felt were of the greatest regulatory importance at the time the handbook was being developed. The four bacterial fish pathogens considered in this chapter represent etiological agents which are known to exist in carrier states, but which have the potential for generating severe epizootics of clinical disease under the appropriate conditions. The methods are described for detection and identification of each pathogen in the absence of clinical signs. While other bacterial pathogens exist which can cause serious disease in fish, they are often widely distributed and cannot be controlled through transfer restrictions due to their ubiquitous nature (such as the pathogenic *Flavobacterium* species), and therefore are not the focus of these inspection procedures.

The accurate identification of a bacterial species is based upon patterns of characteristics observed when live, pure bacterial isolates are cultured under a variety of environmental and biochemical conditions. All four bacterial fish pathogens considered for identification during a Fish Health Inspection are culturable. All have been exhaustively characterized in a variety of widely recognized bacteriological manuals (Bergey's, 1984; MacFaddin's 1980 & 2000; Austin & Austin, 1987). The extensive characterization of these species has lead to the establishment of simple testing schemes for presumptive identification of bacteria isolated from fish tissues as described in these protocols. *Renibacterium salmoninarum ,* however, is relatively fastidious and difficult to culture and characterize phenotypically in the period of time desired to accomplish the completion of a Fish Health Inspection. Serological techniques are also considered to be rapid, highly specific means for achieving presumptive identification of bacteria. Because of its fastidious nature, the fluorescent antibody technique has been long developed as a presumptive screening tool for the detection of *R. salmoninarum* in fish tissues.

It is generally agreed that identification of a bacterial isolate based on phenotypic or serological characteristics alone poses the possibility that a population of fish be inaccurately labeled as diseased on a Fish Health Inspection Report. Although either method of identification is acceptable as a screen for pathogens in fish, neither technique alone is precise enough to distinguish between some similar organisms. For these reasons, it is always necessary to apply a second testing regime, referred to here as "confirmatory", to establish the accuracy of the screening test. The protocols described in this document are presented in such a manner. In past decades, studies with nucleic acids and genetic methods have furthered the accuracy in the classification and identification of bacterial species. These tools, however, were limited to research because of the difficulty in applying them accurately under clinical situations. The more recent developments in polymerase chain technology, however, have revolutionized the use of molecular biology in pathogen detection in clinical laboratories. PCR is a practical, sensitive and accurate means to confirm the presumptive identification of a bacterial pathogen by the isolation and amplification of

segments of DNA existing within fish tissues. It is presented in these protocols as an alternative to time consuming selective culture for confirmation of positive *R. salmoninarum* FAT results.

A3.4 Virology

A. 2000 – 2002 (Initial Position Statement)

Position Statement

The eight viral pathogens considered in this chapter represent agents that may exist in a carrier state, have the potential for causing severe epizootics and/or are currently of regulatory concern. This list will likely change as these concerns vary and new control measures are developed. Techniques provided for screening and confirmation are considered to be sensitive, practical, and efficient, and applicable to the large numbers of samples necessary to detect viral pathogens in carrier states. The potential variety of techniques is limited to cell culture for screening and serum neutralization and/or PCR for confirmation to simplify the writing of this initial Handbook. Other serological methods such as immunoblot and fluorescent antibody tests are available for some of these viruses and applications may be made to add these to later versions.

Cell culture is the screening method used and broad spectrum cell lines have been chosen whenever possible to aid the testing laboratory in getting the most information from the samples.

Blind passage of samples has been included to determine if it will significantly increase the ability of the laboratories to detect carrier stages of these viruses using these methods.

Since cell culture amplifies the virus, it allows for the use of a highly sensitive but not necessarily specific confirmation method (see Chapter 1). The utility of serum neutralization tests for the confirmation of IHNV, IPNV, SVCV, and VHSV has been shown with years of use and for that purpose it is included here, however, the reagents are not available for all of the viruses in this Handbook. PCR is a newer technique that is also highly specific but much more rapid than serum neutralization and the detailed methods for using it to confirm IHNV, ISAV, LMBV, and VHSV are also included. PCR techniques are being developed for IPNV, OMV and WSHV and applications may be made to include them in future version as the methods and reagents become available.

A3.5 Parasitology

A. 2000 – 2002 (Initial Position Statement)

1. Position Statement

The pathogens selected were those the committee felt were of the greatest regulatory importance at the time the handbook was being developed. Rationale for selection of the screening and confirmatory assays for each of the fish parasites considered in Chapter 5 are detailed below. Confirmatory procedures will only be used if the sample is presumptively positive using the approved screening method. Please refer to chapter 1 for explanation of the acceptance of non-validated procedures for confirmation.

Myxobolus cerebralis

Screening - The pepsin-trypsin digest procedure was selected as the assay of choice for isolation and concentration of spore stages from fish cartilage. Although it was acknowledged that the plankton centrifuge method offers some advantages in the ease of assay performance, review of the literature and of laboratories performing *M. cerebralis* diagnostics supported selection of the digest assay for reasons of increased sensitivity. The procedure does allow pooling of up to 5 fish, which is likely to decrease detection sensitivity. However, it was considered that processing of individual fish would constitute a workload beyond the capability of many laboratories, and that in some regions of the country this would be considered unacceptable. The decision was to allow pooling with the realization that in areas most affected by the parasite there would be requirement by the states to process single fish.

Confirmation – Confirmation is either by identification of spores in histological sections or detection of parasite DNA by polymerase chain reaction (PCR) assay. Detection in histological sections is the current standard. Although the committee felt that it is of lower sensitivity than the PCR assay, it will remain an acceptable confirmatory tool at this time. For DNA detection, the nested PCR assay was selected because it scientifically acceptable and citable and it is used successfully in a number of laboratories. Because the sampling and preparation procedures described in the original publication were primarily for research purposes, the protocol described here references methods more in line with those required during field collections of fishes of different sizes. These collection and preparation methods are compatible with performing the nested assay.

Ceratomyxa shasta

Screening – Presumptive identification is based on identification of any parasite stages in wet mount scrapings, the procedure currently recommended.

Confirmation – Because of the distinctive morphology of the *C. shasta* spore, its identification is sufficient for confirmation. If spores are not identified, a

presumptive positive can be confirmed by detection of parasite DNA by PCR. The protocol described is published and has been developed for diagnosis in field situations. Other confirmatory procedures requiring monoclonal antibodies were not considered because these reagents are not commercially available.

Tetracapsula bryosalmona

Screening – Presumptive identification is made by identifying any parasite in stained imprints or using lectins. These two methods were proposed because identification of the parasite is difficult without practice, and the lectin has been shown to increase detection.

Confirmation – At this time, confirmation is by identification of any parasite stages in histological sections. Although this method is not highly sensitive and requires a trained eye, it was agreed that scientific review of other methods made them unfeasible at this time. The lectin stain has been demonstrated to cross-react with other myxozoans and there is also question about the specificity of published PCR assays. The committee felt that this protocol would probably be updated in the near future as a demonstrated specific PCR assay becomes available.

Bothriocephalus acheilognathi

Screening – Presumptive identification is by identification of basic characteristics of the cestode.

Confirmation – Presumptive cestodes are confirmed by identification of key morphological characteristics. These visual identification methods are accepted in the scientific literature and are the current Bluebook standard.

A3.6 Polymerase Chain Reaction – General Protocols

A. 2000 – 2002 (Initial Position Statement)

Position Statement

This chapter was designed to supplement references to molecular techniques referenced in earlier chapters. Included are general considerations for insuring that contamination does not occur and to insure the integrity of the assay. These general protocols that can be found in many general primers for PCR and are intended to provide background information for laboratories that are just setting up PCR diagnostics.

A3.7 Appendix 1

A. 2000 – 2002 (Initial Position Statement)

Position Statement

This Appendix is truly the heart of this document. It lays out the structure of the handbook and the revision and oversight committee. It explains how the handbook will be maintained and by who. Most importantly, however, this Appendix details the manner in which this handbook shall be reviewed and revised. This detailed procedure is what gives this document its advantage over previous documents of its kind. Additionally, these reviews are mandatory on an annual basis, which means the document can be kept current from a technique and pathogen standpoint, such that in the future there should be no need to create any new handbooks or manuals.